Seasonal Influences on In Vitro Fertilization and Embryo Transfer

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Purpose: *The study was aimed to investigate the influence of time factors (day, week, month) on pregnancy rates and in vitro fertilization (IVF) and embryo transfer (ET) parameters.*
Methods: *8184 IVF–ET cycles, taking place in two IVF centers from 1992 to 1999, were analyzed. Multiple logistic and linear regression methods were performed as statistical analyses.*
Results: *Oocyte pickup on Tuesdays achieved a significantly higher mean number of oocytes (median: 7) and the highest pregnancy rate (PR) (33.4%) per ET. There was a significant variation over the year, with the lowest PR/ET in July (25.71%) and the highest in December (35.5%). Concerning outcomes, the age factor and the number of embryos transferred had the highest significant* ($p < 0.0001$) *influence.*
Conclusions: *The observed weekly rhythm for oocyte pickups is certainly due to preprogrammed ovarian stimulation used in our IVF programs. Age as well as the number of embryos transferred are the main influencing factors on a positive outcome and more predictive than seasonal aspects.*

KEY WORDS: In vitro fertilization–embryo transfer; IVF–ET; pregnancy rate, season.

INTRODUCTION

Ever since successful in vitro fertilization (IVF) and embryo transfer (ET) exist, both scientists and biologists keep wondering about the alternating periods of excellent success and periods when only low pregnancy rates are achieved. The latter cannot always be due to technical failures or by changed laboratory or culture conditions. Seasonal rhythms have been suspected to influence both natural and assisted human reproduction.

Several authors (1,2,3) described variations on their success rates, but only one study (4) on a small number of patients definitively proved the significant correlation of such changes with seasonal rhythms.

We decided to analyze our large patient material (8185 IVF–ET cycles) concerning both ooccytes retrieved by Ovum Pick Up (OPU) and pregnancy rates (PRs) per ET, including information on weekly, monthly, and seasonal rhythms.

MATERIAL AND METHODS

Eight thousand one hundred eighty five IVF-ET cycles, taking place in two clinics (Vienna, Austria and Budapest, Hungary) from January 1, 1992, to April, 1999, were analyzed.

Ninety-four percent of the stimulation protocols were preprogrammed with a contraceptive pill and in all cases the first injection (FSH (follicle stimulating hormone) and/or hMG (human menopausal gonadotropin)) was given on a Sunday together with or without a 5-day course of Clomiphen citrate.

For the statistical evaluation, number of oocytes retrieved was transformed by taking the square root in order to reduce the skewness of the distribution. Multiple linear regression was applied to the transformed variable using the influence factors age and number of previous IVF-ET attempts per patient and their squares.

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Table I. Weekly Rhythm of ($n = 8184$) Oocytes Retrieved (Mean ± SD), Median Number of Oocytes Retrieved, and Pregnancy Rates per Embryo Transfer ($n = 7572$) in Percent

	Mean no. of oocytes ± SD ($n = 8184$)	Median no. of oocytes	PR/ET (%)	n ($n = 7572$)
Monday	6.55 ± 4.09	6	27, 3	443
Tuesday	7.45 ± 4.37	7	33, 4	1891
Wednesday	6.80 ± 3.99	6	30, 9	1825
Thursday	6.43 ± 3.83	6	30, 8	1430
Friday	6.19 ± 3.17	5	27, 6	1133
Saturday	5.77 ± 3.76	5	30, 9	521
Sunday	6.21 ± 4.16	5	33, 1	329

Note. SD: standard deviation; PR: pregnancy rate; ET: embryo transfer.

Harmonic sinusoidal trends with weekly and yearly periods for oocyte pick up were included in the independent variables (SAS; Proc. Reg. (5)). A harmonic sinusoidal trend for a certain period length was analyzed in the standard way by adding two regressor variables into the multiple regression model: $\cos(\lambda t)$ and $\sin(\lambda t)$. Here t is the time (in days) starting with January 1, 1992; $\lambda = 2/l$, where l is the period length (e.g., 7 for the sinusoidal trend over a week). The coefficients estimated for these two regressor variables determine the amplitude of the harmonic and the location of its maxima and minima.

The dichotomous variable "pregnancy yes/no" was defined by a positive fetal heartbeat in ultrasound at 8 weeks of gestation. It was analyzed by multiple logistic regression (SAS, Proc. Logistic (5)) using the same influence factors as before, additionally including the number of transferred oocytes and its squares.

Cycles with no transferred oocytes were excluded from the calculations concerning the outcome.

Institutional review board (IRB) was not necessary, since patients were treated as routine and analysis was retrospective.

RESULTS

Number of Oocytes Retrieved

A clear weekly influence could be found ($p < 0.0001$). On Tuesdays and on Wednesdays most Ovum Pick Ups (OPU) (24.8 and 24.2% respectively) took place. Especially on Tuesdays a higher number of oocytes (median: 7) could be retrieved; this trend exists after accounting for age and number of IVF attempts (Table I).

There was a harmonic trend over the year ($p = 0.008$ for the cosine term), with a slightly lower number of oocytes retrieved in early summer (Table II).

No ET could take place in 628 (7.67% of 8185) cycles, because of failed fertilization or slow cleavage of the embryos or a thin (7 mm) endometrium layer. Sixty-four percent of those cycles without ET were in

Table II. Monthly Rhythm of ($n = 8184$) Oocytes Retrieved (Mean ± SD), Median Number of Oocytes Retrieved, and Pregnancy Rates per Embryo Transfer in Percent

	Mean no. of oocytes ± SD ($n = 8184$)	Median no. of oocytes	PR/ET (%)	n ($n = 7572$)
January	6.8 ± 3.9	6	29, 3	583
February	6.9 ± 3.9	6	35	577
March	6.7 ± 4.3	6	31, 2	690
April	6.7 ± 4.0	6	32, 8	674
May	6.6 ± 4.2	6	28, 3	665
June	6.3 ± 3.9	6	30, 3	680
July	6.5 ± .7	5	25, 7	616
August	6.9 ± 4.1	6	29, 7	620
Sept.	6.9 ± 4.3	6	33, 3	564
Oct.	6.9 ± 4.3	6	29, 5	685
Nov.	6.7 ± 4.1	6	31, 9	697
Dec.	6.6 ± 3.9	6	35, 3	521

Note. SD: standard deviation; PR: pregnancy rate; ET: embryo transfer.

Table III. Rhythm of Oocytes (Mean ± SD) and Median Number of Oocytes Retrieved, and Pregnancy Rates per Embryo Transfer in Percent According to Age (Group I–VII) and According to the Number of Attempts (1–4)

	Groups of age						
	I (<20)	II (21–25)	III (26–30)	IV (31–35)	V (36–40)	VI (41–45)	VII (>46)
Mean no. of oocytes ± SD ($n = 8185$)	6.14 ± 2.9	7.21 ± 4.1	7.39 ± 4.1	7.16 ± 4.3	6.1 ± 3.7	4.64 ± 3.0	3.75 ± 2.6
Median no. of oocytes	6	7	7	6	5	4	2
No. of attempts							
1	76, 32	64, 18	61, 5	54, 76	47, 72	41, 87	37, 5
2	15, 79	26, 03	24, 78	26, 07	26, 58	26, 4	20, 83
3	2, 63	5, 93	9, 04	11, 52	13, 55	14, 69	20, 83
4	5, 26	3, 87	4, 67	7, 64	12, 16	17, 03	20, 84
PR/ET (%)	28, 6	33, 8	36, 5	34, 1	26, 4	16	0
n ($n = 7573$)	35	364	1843	2657	1981	674	19

a first, 20.9% in a second, 10% in a third, and 3.3% in a fourth attempt, decreasing to 0.3% in a seventh try.

In 10.9% of cycles only one embryo could be transferred. In 22.9% two, in 39.6% three, in 15.5% four, and in 2.8% five or more embryos were transferred (Table IV).

Age and its square root had a highly significant (both $p < 0.0001$) influence. Comparing seven groups of age (Group I: 20 years and younger; II: 21–25 years; III: 26–30 years; IV: 31–35 years; V: 36–40 years; VI: 41–45 years; VII: 46 years and older) the mean number of oocytes retrieved was 7.21 ± 4.08 (SD (Standard deviation)) and 7.39 ± 4.11 in women of Groups II and III, respectively. These numbers decreased both for ages above Group III and below Group II (Table III).

The number of oocytes retrieved rose slightly with the number of IVF-ET attempts and dropped for more than 5 attempts (Table IV).

Including the twofold interaction between age and number of attempts, the results did not show a significant contribution to the regression.

Outcome

The highest PR/ET (33.4%) could be achieved (Table I) if OPU took place on Tuesdays (24.2% of all OPU). However, the harmonic trend over days of the week did not show any statistically significant influence.

The highest average PR/ET calculated for each month was in December (35.3%), even though the number of IVF-ET attempts (6.73%) in this month was the lowest throughout all the years (Table II). On the other hand in July only 25.7% of women became pregnant.

The number of embryos transferred was predictive for a positive outcome (both, positive term: all

Table IV. Relationship Between the Number of IVF–ET Attempts (I–VII) and Number of Embryos Transferred (1–6), Pregnancy Rates/Embryo Transfer per Attempt With an ET and PR per Number of Embryos Transferred in Percent

		Number of IVF–ET attempts							PR/No. of embryos transferred (%)
		1	2	3	4	5	6	7	
No. of oocytes retrieved ± SD ($n = 8185$)		6.58 ± 4.1	6.82 ± 4.0	6.88 ± 4.1	6.72 ± 3.9	7.01 ± 4.1	6.42 ± 3.9	6.58 ± 3.2	
No. of embryos transferred									
0	628	9, 11	6, 2	6, 7	5, 5	2, 8	4, 8	2, 4	—
1	894	11, 7	10	10, 2	9, 7	6, 7	12	9, 5	16, 11
2	1818	24, 5	22, 1	18, 3	21, 3	23, 6	15, 7	22, 6	27, 26
3	3247	39, 81	39, 3	38, 7	38, 7	39, 9	50, 6	39, 3	33, 85
4	1274	13, 2	18, 9	18, 4	17, 1	15, 1	12	20, 2	37, 52
5	231	1, 4	3, 1	6, 5	5, 8	9	2, 4	1, 2	39, 83
6	49	0, 2	0, 4	1, 3	1, 8	2, 8	2, 4	4, 8	35, 53
n	8201	4414	2121	941	380	178	83	84	
PR/ET (%)		32	31, 7	29, 8	25, 9	23, 7	22, 8	14, 6	
n	7573	4012	1990	878	359	173	79	82	

$p < 0.0001$; and negative quadratic term: all $p = 0.0007$) (Table IV).

Age and its square root again had a highly significant ($p < 0.0001$) influence. In Group III (26–30 years) an average PR of 36.5% was achieved (Table III), decreasing by age and in the groups below Group III.

A first and a second IVF-ET treatment achieved a PR of nearly 32% each, a third 29.8%. The PR continually decreased to 14.6% for a seventh attempt (Table IV).

In Group I, 76.3% of all IVF-ET attempts have been the first for the woman. With increasing age the percentage of first attempts decreased (Group II: 64.2; III: 61.5; IV: 54.8; V: 47.7; VI: 41.9; VII: 37.5) while the percentage of more than two attempts increased (Table III).

The number of attempts was not significant as a predictive factor ($p > 0.4$) for the outcome. Obviously the frequency of pregnancy decreases with the number of previous attempts, but the number of attempts also clearly increases with age. The number of attempts and the number of transferred embryos did not show a significant relationship (i.e., a larger number of embryos transferred in later attempts) (Table IV).

Including twofold interaction between the number of attempts, age, and number of transferred embryos did not lead to a noticeable change in the results of the logistic regression analysis.

DISCUSSION

A clear influence of the seasons on the outcome of IVF-ET was found by some authors (1,2,3,4). Our data include 8185 IVF-ET cycles, the highest number published so far concerning temporal influences. To analyze the influencing parameters we took the date of OPU, because this is equivalent to the time of ovulation, while the date of ET ranges between the first and the sixth day after OPU.

The results show a trend to better PRs in the colder months: September–April (average PR about 32.2%) versus May–August (28.5%). This is in accordance with both Stolwijk *et al.* (1) and Fleming *et al.* (2). Seasonal variations in sperm parameters may be the cause of different PRs during the year and this has aroused the interest of many authors before: In a retrospective study Levine *et al.* (6) found significantly lower sperm concentrations and motile sperms during the summer months. Similar results were detected later in the quality of semen in outdoor workers (7). Significant seasonal variations in semen parameters of donor specimens (percent motility, motile count, percent rapid sperm, and VSL) were also reported to be lowest in summer time by Centola *et al.* (8). Concerning the results of andrology patients, the sperm count was highest in spring and winter, although the difference was not significant. Reinberg *et al.* (9) failed to find significant variations in sperm parameters, but their results also showed peaks in different seasons. The sperm count/mL was highest in April and June and sperm motility had the highest values in November and December in 260 men before vasectomy. This rhythm with (more or less) better results for the characteristics of semen specimens in the colder seasons is in accordance with the findings of many other authors (10,11,12,13,14).

These differences in sperm quality during the year, as mentioned above, could have influenced fertilization as well as pregnancy rates, particularly during the years when Intracytoplasmic Sperm Injection (ICSI) did not yet come into existence.

On the other hand, melatonin has been demonstrated to vary seasonally in follicular fluid (15), with higher concentrations in daytime values in the dark seasons (16,17). It is suggested that melatonin may be involved in the steroid genesis in human ovaries (18,19), and so it seems to be possible that melatonin is an additional seasonally influenced–and influencing–factor for IVF-ET.

Although we tried to keep the conditions as constant as possible over the year, it cannot be completely excluded that the staff performance or the laboratory environment was influenced by seasons. However, we did not see any relevant changes, e.g., when looking at data of the persons doing the OPU.

Randomization of cycle starts and egg retrievals is not possible in such a clinical setup. However, we accounted for systematic changes over the days of the week by including a sinusoidal trend over the days of a week.

The observed weekly rhythms (more oocytes retrieved at the beginning of a week and best PR on Tuesdays) are certainly due to preprogrammed ovarian stimulation used in our IVF programmes. Good responders with many oocytes had OPU on Tuesdays; poor responders with a prolonged stimulation period had a lower number of oocytes retrieved on Saturdays and Sundays.

Finally, we may conclude that parameters like age and number of embryos transferred have a stronger influence on a positive outcome than any temporal aspects.

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